

Attorney Docket No.: PENN-0583
Inventors: Lee and Doms
Serial No.: 09/297,877
Filing Date: June 28, 1999
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In the Specification:

Please amend the specification as follows:

Paragraph beginning at line 1 of page 15 has been amended as follows:

**Example 2 Metabolic Labeling, Gel Electrophoresis, Immunoblotting
and Quantitation**

Cultured NT2N neurons were starved in methionine-free DMEM HG (Life Technologies, Inc., Gaithersburg, MD) for 30 minutes prior to incubation in fresh methionine-free DMEM HG containing 0.5 mCi/ml of [³⁵S]methionine (sp act. 1000 Ci/mmol; NEN-DuPont, Boston, MA). For steady-state labeling studies, NT2N neurons were labeled with [³⁵S]methionine continuously for 16 hours. For pulse-chase studies, cells were labeled with [³⁵S]methionine for 1 hour, washed twice with methionine-containing DMEM, and then chased in the same medium for 0 to 24 hours. APP_{FL}, APP α and APP β were separated on 7.5% Laemmli SDS-PAGE gels, and A β and p3 were separated on 10/16.5% step-gradient Tris-Tricine gels. These gels were either stained with Coomassie Brilliant Blue R (Pierce, Rockford, IL) and dried or transferred to nitrocellulose membranes and dried prior to exposure on PhosphorImager plates (Molecular Dynamics Inc., Sunnyvale, CA) for 3-5 days. The nitrocellulose replicas containing the immunoprecipitates were further probed with different antibodies in

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accordance with procedures described by Wertkin et al. 1993 *Proc. Natl. Acad. Sci. USA* 90:9513-9517. Quantitation of bands in the autoradiogram was performed using the IMAGEQUANT software (Molecular Dynamics Inc. Sunnyvale, CA) in accordance with procedures described by Turner et al. 1996 *J. Biol. Chem.* 271:8966-8970. Radiolabeled proteins in SDS-PAGE gels and nitrocellulose replicas were also analyzed by standard autoradiographic methods. All experiments were repeated between 3 and 6 times.

Paragraph beginning at line 29 of page 15 has been amended as follows:

Example 3 Sample Preparation and Serial Immunoprecipitations

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Cell lysates were prepared in accordance with procedures described by Golde et al. 1992 *Science* 255:728-730. Protein concentration was determined by the bicinchoninic acid procedure (Pierce, Rockford, IL). Media were centrifuged at 100,000 x g for one hour at 4°C before immunoprecipitation. Both cell lysates and media were precleared with protein A-SEPHAROSE (Pharmacia Biotech, Piscataway, NJ) in RIPA for one hour at 4°C. After recentrifugation at 15,000 x g for one minute, the supernatants were rocked overnight at 4°C with fresh protein A-SEPHAROSE and the appropriate primary antibody. After collecting the immunoprecipitates by recentrifugation at 15,000 x g for 1 minute,